Alterations in the intestinal microbiome associated with PCOS affect the clinical phenotype

Lan Wang[a,b,c], Jing Zhou[a,b,c], Hans-Jürgen Gober[d], Wing Ting Leung[a,b,c], Zengshu Huang[a,b,c], Xinyao Pan[a,b,c], Chuyu Li[a,b,c], Na Zhang[a,b,c,*], Ling Wang[a,b,c,*]

[a] *Laboratory for Reproductive Immunology, Hospital & Institute of Obstetrics and Gynecology, Shanghai Medical College, Fudan University, Shanghai, China* [b] *The Academy of Integrative Medicine, Fudan University, Shanghai, China*
[c] *Shanghai Key Laboratory of Female Reproductive Endocrine-related Diseases, Shanghai, China* [d] *Department of Pharmacy, Neuromed Campus, Kepler University Hospital, 4020 Linz, Austria*

ARTICLEINFO

Keywords:
PCOS
Intestinal microbiome

ABSTRACT

Polycystic ovarian syndrome (PCOS), characterized by chronic anovulation and hyperandrogenaemia, is a complex endocrine and metabolic disorder commonly seen in women of reproductive age. Multiple factors, including the intestinal microbiome, affect the pathogenesis and development of PCOS. However, the specific mechanisms by which gut microbes play a role in PCOS remain elusive. This review summarizes recent research about the transformational changes in gut microbes revealed in PCOS patients and the possible mechanisms and pathways by which the intestinal microbiome exerts influence on PCOS progression and phenotypes. In addition to the intestinal microbiome, evidence from animal studies suggests changes in the vaginal microbiome under PCOS conditions. The alteration of microbiome could affect oestrus cycle and PCOS phenotypes. Microbiome is closely associated with medicine and therapeutic approaches. Microbiome influences drug and therapy response and itself is a new source of therapy. Accurate modulation of the intestinal and vaginal microbiome is a potential therapy for PCOS patients. Future studies are required to elucidate the specific role of each particular genera of microbiota and the mechanism by which microbiome impacts the pathogenesis, progression and phenotypes of PCOS.

1. Introduction

Polycystic ovary syndrome (PCOS) affects 6 %–10 % of reproductive aged women [1] and is often accompanied by insulin resistance, obesity and infertility. However, the exact pathogenesis of PCOS is presently unknown because of heterogeneity. Some studies indicated that composition of gut microbes was associated with sex hormones [2,3], and Shermel B. Sherman also reported that prenatal exposure to excess androgen led to gut microbial dysbiosis [3]. The intestinal microbiome may have bidirectional interactions with PCOS. Gut bacteria play a role in the pathogenesis of PCOS, and PCOS conditions lead to composition changes in the gut bacteria as well. Many studies have reported alterations of enteric microorganisms in women with PCOS as well as in rat and mouse models of PCOS [2,4]. Previous research also suggested a new hypothesis that the dysbiosis of gut microbiota accounted for the development of PCOS [5]. Researchers have proposed several pathways by which some particular species are related to PCOS phenotypes, but the specific mechanism of microbe-PCOS interaction remains elusive, thus limiting the search for potential therapies. This review provides an overview of research related to the microbiome and PCOS to this point. **2. Methodology**

We searched the PubMed for pertinent literatures in recent ten years with ‘intestinal microbiome’ and ‘polycystic ovary syndrome’ as key words. We included articles that reported the alterations of intestinal microbiota under PCOS condition and divided them into three categories according to cohort types: PCOS mouse model, PCOS rat model and PCOS patients. In addition, we included articles that explored and discussed the mechanisms by which intestinal microbiome influenced the pathogenesis, progression and phenotypes of PCOS. These articles can be divided into four categories according to the association they put forward between PCOS and microbiome, which was summarized in Table 1. Moreover, we searched the PubMed for pertinent literatures with ‘vaginal microbiome’ and ‘polycystic ovary syndrome’ as key words to include articles that revealed the alterations of vaginal microbiota under PCOS condition.

3. Polycystic ovarian syndrome

PCOS is an endocrine and chronic metabolic disease in women [5]. Compared with healthy controls, women with PCOS have higher levels of serum luteinizing hormone (LH) and androgens, while their levels of serum follicle stimulating hormone (FSH) and sex hormone binding globulin tend to be lower [6].

Due to the heterogeneity within PCOS, there are no unified diagnostic criteria for PCOS. Currently, there are three widely accepted diagnostic criteria: the NIH criteria, the Rotterdam criteria and the Androgen Excess-PCOS Society criteria [7].The NIH criteria, presented at a meeting sponsored by National Institutes of Health (NIH) in 1990, defined PCOS as hyperandrogenism and/or hyperandrogenaemia with oligo-anovulation excluding similar disorders. The Rotterdam criteria in

Abbreviations: PCOS, polycystic ovarian syndrome; LH, luteinizing hormone; FSH, follicle stimulating hormone; NIH, National Institutes of Health; AES, Androgen Excess Society; SCFAs, short chain fatty acids; GDCA, glycodeoxycholic acid; TUDCA, tauroursodeoxycholic acid; IL-22, interleukin-22; GATA3, GATA binding protein 3; PYY, peptide YY; LPS, lipopolysaccharide.

* Corresponding authors at: Obstetrics & Gynecology Hospital of Fudan University, 419 Fangxie Road, Shanghai, 200011, China. *E-mail addresses:* zhangna_shmu@163.com (N. Zhang), Dr.wangling@fudan.edu.cn (L. Wang).

https://doi.org/10.1016/j.biopha.2020.110958
Received 17 July 2020; Received in revised form 1 October 2020; Accepted 27 October 2020
Available online 7 November 2020
0753-3322/© 2020 The Authors. Published by Elsevier Masson SAS. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

2003 recommended the diagnosis of PCOS be based on at least two of these features: 1) clinical or biochemical hyperandrogenism (Ferriman-Gallwey score ≥8; testosterone level >0.5 ng/mL) [8]; 2) oligo-anovulation; 3) polycystic ovaries. The overall prevalence of PCOS varied from 6% according to the NIH criteria to 10 % when applying the Rotterdam or AE-PCOS Society criteria [1]. A report by the Androgen Excess Society (AES) stated that PCOS was primarily a disorder of hyperandrogenism [9], and thus women with polycystic ovaries and oligo-anovulation but without demonstrable androgen excess were excluded [7]. International paediatric subspecialty societies also reached a consensus for the diagnosis of PCOS in adolescents: persistent hyperandrogenic oligo-anovulatory menstrual abnormality based on age- and stage-appropriate standards [10].

Obesity, one of the major risk factors for PCOS, seems to exacerbate PCOS phenotypes, while diet and exercise may alleviate PCOS symptoms. PCOS phenotypes vary with ethnicity, region, age and weight. Furthermore, PCOS confers a substantially increased risk for insulin resistance, type II diabetes, obesity, infertility [11], cardiovascular disease, hypertension [12,13] and psychological illness [14]. Further researches aiming to explore the clinical characteristic of PCOS patients is required.

There are many risk factors for PCOS and the pathogenesis of PCOS is complicated. Current research has revealed some correlations between intestinal microbiome and PCOS.

4. Intestinal microbiota

Intestinal microflora are symbiotic microorganisms living in the intestinal tract of human beings. According to the flora size, intestinal microbiota can be divided into predominant microflora and subdominant microflora. The former refers to bacteria with high population density generally above 10 ~ 10 Cfu /g, mainly including obligate anaerobic bacteria such as *Bacteroides*, *Eubacterium*, *Bifidobacterium*, *Ruminococcus* and *Clostridium*. While subdominant microflora consists of aerobe or facultative anaerobe bacteria. The population density and diversity of microbiota are highest in the colon.

Gut microbes are closely related to human health from many aspects. Intestinal microorganisms produce short chain fatty acids (SCFAs), which play an important role in immunomodulation [15,16] such as the reversal of lung inflammation [17]. Alterations in the composition of gut microbes have an impact on liver function, and chronic liver diseases are often accompanied by gut microbe dysbiosis. Therefore, modulation of gut microbes may be a potential therapy for liver disorders [18]. The gut microbiota also plays a role in age-associated muscle decline, alterations in muscle size and the composition and function of the ageing skeleton [19]. Metabolites generated by gut microbiota, such as bile acids, may contribute to the pathogenesis and development of diabetes and obesity [20,21]. Most importantly, the main function of gut bacteria is to help the host digest quantities of dietary substrates such as carbohydrates, proteins, phytochemicals and the like. The effective uptake, absorption and utilization of metabolites are essential for body material exchange and energy metabolism [22].

Furthermore, the intestinal microbiota is considered to be associated with cardiovascular disease, cancer, cognitive function and gynaecopathy such as endometrial hyperplasia, endometriosis, PCOS and infertility by regulating circulating oestrogen levels [23]. In conclusion, the intestinal microbiome contributes significantly to lesion of various organs or tissues and plays an essential role in normal body functioning. Current research has also revealed an association between PCOS and changes in the gut microbiome.

5. Intestinal microbiota is closely associated with PCOS symptoms

5.1. Alteration of intestinal microbiota under PCOS conditions

Changes in gut microbiota composition have been found in animal models and clinical studies between PCOS conditions and control groups, including alterations in bacterial diversity and the abundance of specific genera.

In the letrozole-induced PCOS mouse model, studies have reported lower abundance of Bacteroidetes [24], *Lactobacillus* and *Christensenella* [25], and higher abundance of most Firmicutes species, including Lachnospiraceae, Erysipelotrichaceae, Ruminococcaceae [24], *Bacteroides vulgatus* [6] and *Coprobacillus* [25]. For the diversity of intestinal microbiota, Qi et al. reported that there was no obvious difference in the α diversity of bacteria, while there was a significant reduction in β diversity between PCOS mice and controls [6]. However, other researchers have reported lower α diversity and alterations in β diversity under PCOS conditions [24,25].

In the letrozole-induced PCOS rat model, the abundance of *Lactobacillus*, *Ruminococcus* and *Clostridium* decreased, while *Prevotella* increased compared with the control group [26]. Additionally, previous works have shown that drug intervention impacted the composition of the gut microbiota [27,28]. One study established a dihydrotestosterone (DHT)-induced PCOS rat model and discovered that Diane-35 (oestrogen and progesterone), an androgen antagonist, increased α diversity in the gut microbes, therefore significantly improving the reproductive and metabolic functioning of PCOS rats [29]. Another study established a dehydroepiandrosterone (DHEA)-induced PCOS rat model. After inulin treatment, *Bifidobacterium* was increased, while *Proteobacteria*, *Helicobacter* and *Parasutterella* were decreased; additionally, *Helicobacter* decreased after metformin treatment [30]. Therefore, in the PCOS rat model, intestinal microbiota was proven to be sensitive to drug treatments.

Gut microbiome composition differs between PCOS patients and healthy controls, as demonstrated in many clinical studies. One study noted that the rare taxa Tenericutes, Bacteroidetes and another unclassified phylum were undetectable in PCOS groups [31]. The abundance of *Porphyromonas* spp., *B. coprophilus*, *Blautia* spp., and *F. prausnitzii* was shown to be higher in women with PCOS, while the levels of *Anaerococcus* spp.,

Odoribacter spp., *Roseburia* spp., and *R. bromii* were lower [8]. Additionally, Insenser et al. stated that women with PCOS had a higher abundance of *Catenibacterium* and *Kandleria* genera [32], and Zeng et al. discovered that the abundance of Bacteroidaceae dramatically rose and that of Prevotellaceae was sharply reduced in PCOS patients, especially in women with both PCOS and insulin resistance [33]. Liu et al. found that the abundance of *Bacteroides*, *Escherichia/Shigella* and *Streptococcus* increased, whereas *Akkermansia* and Ruminococcaceae decreased in PCOS patients [34]. The abundance of *Faecalibacterium, Bifidobacterium* and *Blautia* was shown to be lower in the PCOS group, while that of *Parabacteroides* and *Clostridium* was higher [35]. With regard to diversity changes, researchers reported lower α diversity [8,31–33] and an alteration [8] or reduction [32] in β diversity under PCOS conditions.

Current research has revealed alterations in various bacterial genera in PCOS rat and mouse models as well as in human patients. Treatments that relieve the symptoms of PCOS are also accompanied by compositional changes in the intestinal microbiota. Researchers have suggested several pathways and possible mechanisms through which gut microbes are associated with PCOS.

5.2. Possible mechanisms through which gut microbes are associated with PCOS

5.2.1. Bile acids and interleukin-22

A human-mouse study conducted by Qi et al. revealed a causal relationship between the microbiome and PCOS.

Researchers have reported a significant increase in *Bacteroides vulgatus* (*B. vulgatus*) in PCOS patients compared with healthy controls. *B. vulgatus* was known to play an important role in the pathogenesis of PCOS; healthy recipient mice transplanted with faecal microbiota from PCOS women or fed *Bacteroides vulgatus* developed PCOS phenotypes such as insulin resistance, a disrupted oestrous cycle, altered ovarian morphology and hormone abnormalities.

Qi et al. observed that the abundance of bile salt hydrolase genes that encode bile salt hydrolases was remarkably increased in *B. vulgatus* in PCOS patients. The highly expressed genes produced plentiful bile salt hydrolases, which led to a dramatic decrease in levels of glycodeoxycholic acid (GDCA) and tauroursodeoxycholic acid (TUDCA) in PCOS groups.

Likewise, alterations of the gut microbiota changed the quantity of cytokines produced by intestinal immune cells. Interleukin-22 (IL-22) mRNA levels of recipient mice were substantially reduced after oral gavage with *B. vulgatus* or treatment with stool from PCOS patients.

It was observed in subsequent experiments that GDCA and TUDCA administration upregulated IL-22 mRNA levels and IL-22 levels in both intestinal and serum. GDCA administration increased the mRNA level of GATA binding protein 3 (GATA3), which was pivotal in the production of IL-22. Therefore, bile acid might upregulate IL-22 production through GATA3 signalling pathway.

In addition, IL-22 administration was very effective in correcting PCOS phenotypes, such as ameliorating insulin resistance, regulating disrupted oestrous cycles, reversing ovary morphological changes and improving infertility. There were two possible mechanisms for these findings: first, IL-22 upregulated the expression of brown fat-related genes like Ucp1, Pgc1a, Cited1 and Cox8b, and brown adipose tissue helped improve many symptoms of PCOS; Second, IL-22 might target ovarian granulosa cells to ameliorate inflammation. Further studies are required to prove these mechanisms and to explore the specific pathways involved [6].

In PCOS patients, an increased abundance of *Bacteroides vulgatus* reduced the concentrations of the bile acids GDCA and TUDCA, which negatively influenced the production of interleukin-22. Decreased IL-22 was not able to function well to reverse the PCOS phenotypes, leading to insulin resistance and ovary disfunction. This research suggests that modification of the gut microbiome and regulation of bile acid metabolism and IL-22 levels are of potential value as therapeutic approaches.

5.2.2. SCFAs and gut-brain mediators

One clinical study by Zhang et al. employed microbiome modification as a therapeutic approach, which resulted in improved lab parameters. The researchers observed that the abundance of beneficial microbes such as *Faecalibacterium, Bifidobacterium* and *Blautia* was remarkably lower in the PCOS group, while that of *Parabacteroides* and *Clostridium* was higher. Additionally, PCOS patients showed lower concentrations of short-chain fatty acids in their faecal samples, which were the main beneficial metabolites of intestinal microorganisms. Furthermore, in PCOS groups, the levels of gut-brain mediators such as peptide YY (PYY) and ghrelin were lower. Ghrelin was a peptide that enhances appetite as well as reduces fat utilization. Ghrelin and PYY both played crucial roles in functions concerning the hypothalamus- pituitary gland-gonad axis. Finally, the concentrations of sex hormones were also higher in the PCOS group.

The administration of *Bifidobacterium lactis* V9 exerted great impact on the composition of the intestinal microbiota of PCOS patients. For the majority of participants, the abundance of *Faecalibacterium*, *Bifidobacterium*, *Butyricimonas* and *Akkermansia* increased, while disease- related genera such as *Collinsella*, *Coprococcus*, *Klebsiella*, *Clostridium*, *Actinomyces*, *Streptococcus*, *Eubacterium* and *Ochrobactrum* decreased. *Bifidobacterium* stimulated innate immunity and contributed to the balance of microbiota. *Faecalibacterium prausnitzii* was also a probiotic with anti-inflammatory properties that produced butyrate to improve gut health [36].

The administration of *Bifidobacterium lactis* V9 also resulted in a notable increase in the levels of intestinal SCFAs and gut-brain mediators (ghrelin and PYY). PCOS patients were found to have higher LH levels and LH/FSH ratios, although with the supplement of probiotics, the level of LH and the LH/FSH ratio sharply decreased.

There was a possible mechanism, therefore, through which the administration of gut probiotics influences PCOS parameters. Probiotics regulated or reconstructed the intestinal microbiome by

competitive exclusion mechanisms. They promoted the growth of *Faecalibacterium prausnitzii*, *Bifidobacterium* and *Akkermansia*, which were SCFA-producing bacteria, and led to an increase in intestinal SCFAs. These SCFAs bound to their receptors on enteroendocrine cell membranes and directly stimulated the release of gut-brain mediators such as ghrelin and PYY. The increase in PYY and ghrelin influenced sex hormone secretion by the hypophysis and hypothalamus through the gut-brain axis, thus exerting an impact on PCOS symptoms [35].The increased production of SCFAs also contributed to the barrier function of the gut and reduced the translocation of endotoxins across the gut wall, resulting in reduced inflammation and insulin resistance.

Another study also discovered an association between gut microbiota and gut-brain mediators. It was found that *Bacteroides*, *Escherichia/ Shigella* and *Streptococcus* were increased, while *Akkermansia* were decreased in PCOS patients. *Bacteroides*, *Escherichia/Shigella* and *Blautia* were negatively correlated with ghrelin, while *Akkermansia* showed positive correlation with ghrelin. Thus, researchers proposed that gut microbes may regulate mediators of the brain–gut axis to impact PCOS phenotypes. Additionally, the study proposed another possible mechanism for PCOS pathogenesis: that bacteria that produced lipopolysaccharides (LPSs) increased, while protective bacteria such as *Akkermensia* decreased [34].

In this regard, the accurate modulation of intestinal microbes, such as through the use of probiotic supplements, shows great potential for clinical application in the improvement of PCOS symptoms.

5.2.3. Sex hormones

A rat study done by Guo et al. reported that, in the letrozole-induced PCOS rat model, the abundance of *Lactobacillus*, *Ruminococcus* and *Clostridium* decreased, while *Prevotella* increased compared with the control group [26].

There is potential bidirectional interaction between sex hormones and gut microbiota. The abundance of *Prevotella* is positively related with androgen levels, especially testosterone and androstenedione [37, 38], and the abundance of *Kandleria* is positively correlated with circulating androstenedione concentrations [32]. *Lactobacilli* are also positively correlated with oestradiol and estrone levels [26]. The interplay between gut microbes and sex hormones, therefore, may contribute to the pathogenesis of PCOS.

Transplantation of *Lactobacillus* or faecal microbiota from normal rats improved the PCOS phenotypes. All rats transplanted with faecal microbiota showed improved oestrous cycles, and 75 % of the rats transplanted with *Lactobacillus* performed reduced androgen biosynthesis. In addition, their ovarian morphologies normalized [26]. This study adopted microbiome modification as a therapeutic approach and proved that microbiome regulation modified PCOS pathology.

In conclusion, current studies have shown that intestinal microbes influence the progression of PCOS by upregulating or downregulating hormone secretion, gut-brain mediators, cytokines, and metabolite production.

The increased abundance of *Bacteroides vulgatus* under PCOS condition reduced the concentrations of the bile acids, subsequently downregulated the production of IL-22, which was protective and effective at ameliorating inflammation and insulin resistance, thus leading to PCOS phenotypes. The increased abundance of *Prevotella* under PCOS condition upregulated androgen levels while decreased abundance of *Lactobacillus* downregulated oestradiol and estrone levels, contributing to androgen excess. In addition, the administration of probiotics improved PCOS symptoms by upregulating the production of SCFAs. Probiotics promoted the growth of *Faecalibacterium prausnitzii*, *Bifidobacterium* and *Akkermansia* that produced SCFAs, then the increased SCFAs stimulated the production of gut-brain mediators to exert an impact on PCOS symptoms.

Thus, modification of the gut microbiome and regulation of metabolites and cytokine IL-22 levels are potential therapeutic approaches for PCOS patients. The association between PCOS and microbiome is summarized in Table 1 and possible mechanisms by which gut microbiome influences PCOS phenotypes are demonstrated in Fig. 1.

The figure summarizes six different pathways or mechanisms to illuminate how the alterations in bacterial diversity and relative abundances of specific genera influence PCOS phenotypes. The pathways represented by dashed lines refer to mechanisms that have not yet been verified.

1 The abundance of *Bacteroides vulgatus* increased, which caused reductions in the concentrations of GDCA and TUDCA bile acids, resulting in a decline in interleukin-22 production. Decreased IL-22 cannot function well to ameliorate PCOS phenotypes, leading to insulin resistance and ovary disfunction.
2 The administration of gut probiotics promoted the growth of SCFA- producing bacteria, therefore increasing intestinal SCFAs. SCFAs stimulated the release of the gut-brain mediators like ghrelin and PYY. Ghrelin and PYY influenced sex hormone secretion through the gut-brain axis, which ameliorated PCOS symptoms.
3 The increases in *Bacteroides* and *Escherichia/Shigella* and the decrease in *Akkermansia* both downregulated ghrelin production. Decreased ghrelin influenced sex hormone secretion through the gut-brain axis, which might exacerbate PCOS symptoms.
4 The abundance of *Prevotella* increased, which led to an increase in the levels of testosterone and androstenedione. Meanwhile, the

Table 1

Association between PCOS and Microbiome.

	Causality of relationship unclear.	Causal relationship.	Microbiome modifies lab parameters.	Microbiome modifies PCOS pathology.
Mouse studies	Kelley et al. [24] Torres et al. [25] Zhang et al. [29]	Qi et al. [6]		
Rat studies	Xue et al. [30] Singh et al. [39] Lindheim et al. [31] Torres et al. [8]			Guo et al. [26]
Human studies	Insenser et al. [32] Zeng et al. [33] Liu et al. [34]		Zhang et al. [35]	

abundance of *Lactobacilli* decreased, leading to decrease in oestradiol and estrone levels. Changes in sex hormone concentrations might contribute to PCOS pathogenesis.

5 LPS-producing bacteria increased, while protective bacteria such as *Akkermensia* decreased. LPS induced metabolic disorders that might lead to obesity and insulin resistance.

6 Bacterial α diversity was decreased, altering the normal function of the gut and leading to PCOS phenotypes.

6. Research on the vaginal microbiome and PCOS

Many studies have revealed the relevance of the relationship between the alteration of enteric microorganisms and PCOS. However, few studies have explored the relationship between vaginal microbes and PCOS. One study [39] conducted by Singh et al. indicated some changes in the vaginal microbiota in rats with PCOS. Cyclical fluctuations of the vaginal microbiota were observed in normal rats, whereas the rats with PCOS did not show these fluctuations. Additionally, the vaginal bacteria count in PCOS rats was remarkably higher than in the control groups, and electron microscopy scanning showed that numerous bacteria adhered to the cervical mucosa in PCOS rats, which was consistent with rats in oestrus. The concentrations of sex hormones including oestradiol and progesterone probably played a role in the modulation of vaginal microbiota, and the influx of leukocytes was negatively associated with the colonization of microbes in the vagina. However, the specific mechanisms and pathways through which sex hormones impact the leukocyte concentrations and vaginal microbiota abundance remain elusive. Whether vaginal microbiota has an effect on sex hormone concentrations and PCOS phenotypes also requires further study.

7. Clinical implications

Current study has shown that microbiome is closely associated with therapeutic approaches of many diseases. First, microbiome can exert an impact on drug and therapy response; second, microbiome itself is a source of new therapy [40].

Microbiome influences drug response and therapeutic effect in different people. Several studies revealed that microbiome impacted efficacy of immunotherapy in melanoma patients. Patients who showed a good response to anti-PD-1 therapy featured higher α diversity and higher abundance of the Ruminococcaceae family in their faecal

microbiome samples, which indicated that patients with favorable gut microbiome can have better therapeutic response and develop greater anti-tumor immunity [41]. Another study done in mice model supported the result and discovered that reconstruction of germ-free mice with faecal material from responsive patients improved the efficacy of anti-PD-L1 therapy [42,43]. In addition, dysbiosis of microbiome resulted in lower efficacy of CpG-oligonucleotide immunotherapy and platinum chemotherapy [44]. Some genera, however, such as Eggerthella lenta, inactivated and reduced the cardiac drug digoxin both in vivo and in vitro [45]. Thus, appropriate modulation of microbiome may be adopted as an adjuvant therapy to achieve the optimal responses of drugs or therapies.

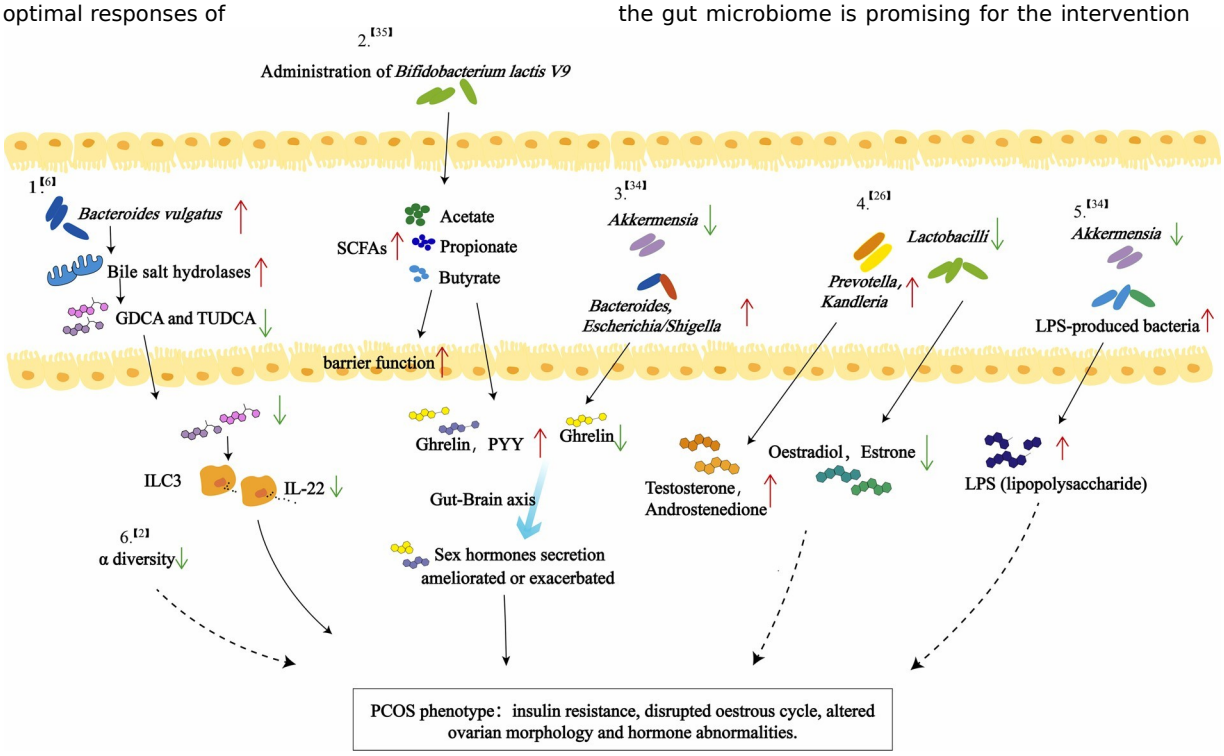

Fig. 1. Pathways and Mechanisms via which Gut Microbiota Influences PCOS Phenotypes.

Furthermore, microbiome itself, is a new therapy with great potential. Faecal microbiota transplantation (FMT) is a therapy that transfers stool from healthy people to patients to repair the disruption of normal microbial communities. FMT has been proved effective in the treatment of recurrent *Clostridium difficile* infection [46]. Besides, another study stated that rectal bacteriotherapy was a feasible alternative to FMT in patients with relapsing *Clostridium difficile* diarrhoea [47]. FMT has also been proved to be a potential therapy for ulcerative colitis [48] and other inflammatory bowel diseases [49]. Besides, tungstate-mediated microbiota editing relieved intestinal inflammation in mouse model of colitis [50]. Autologous FMT was proved better than just probiotics transplantation in the reconstitution of gut microbiome after antibiotics-associated dysbiosis [51]. In addition, nanotechnology was also promising to be used to manipulate microbiome for cancer treatment [52].

Using microorganisms that are genetically engineered is also a novel perspective. *Escherichia coli*, which was engineered to express genes encoding Phe-metabolizing enzymes, was a potential therapy for phenylketonuria [53]. Another study also engineered *Escherichia coli* to kill *Pseudomonas aeruginosa* or inhibit their virulence to prevent and reduce its infection. The efficacy has been proved both in vitro experiment and in animal models of caenorhabditis elegans and mice [54]. Besides, administration of *Lactococcus lactis* modified to secrete proinsulin autoantigen and immunomodulatory cytokine IL-10 was proved to reverse autoimmune diabetes in mice model, which may be potentially effective for type I diabetes in humans.

Microbiome-directed or microbiome-associated therapies are variable in forms. The modulation of the gut microbiome is promising for the intervention of many circulating oestrogen-related diseases, such as obesity [23], metabolic syndrome [55], endometriosis [56] and infertility [57].

The close association between PCOS and the intestinal microbiome also provides potential therapies for PCOS. PCOS rats treated with faecal microbiota transplantation from healthy rats showed improved oestrous cycles, and androgen biosynthesis decreased in PCOS rats transplanted with *Lactobacillus* [26]. Zhang et al. also reported that the administration of *Bifidobacterium lactis* V9 on PCOS patients upregulated beneficial intestinal microbes and downregulated disease-related genera [35]. Finally, after Diane-35 or inulin treatments, PCOS rats showed functional restoration accompanied with changes in the gut microbiome [29].

Current research has revealed close correlations between PCOS phenotypes and the intestinal microbiome. Modulation of the microbiome is potentially a new therapy for PCOS patients; while most recent studies have been conducted in PCOS rat models, more studies on PCOS patients are needed for further exploration.

8. Conclusion

Enteric as well as vaginal microorganisms are closely associated with PCOS pathogenesis and phenotypes. PCOS patients have altered microbiota compositions and reduced microbial diversity. Additionally, intestinal microbes influence the phenotypes of PCOS patients by upregulating or downregulating hormone secretion, gut-brain mediators and metabolite production.

Limitations also exist in the current research of this topic. Present studies have not answered the question of cause and effect. Alterations in the microbiome may be the cause of PCOS, they may only be a prodromal symptom without causal relationship, or they may be the consequence of PCOS with reciprocal interactions. The intrinsic mechanisms are still unclear.

Modulation of the microbiome is a potential therapy for PCOS patients. Future studies will be required to elucidate the specific mechanisms by which the microbiome impacts the pathogenesis, progression and phenotypes of PCOS.

Declaration of Competing Interest

The authors report no declarations of interest.

Acknowledgements

Funding: This work was supported by the 2018 Program to Guide Medicine ("Yixueyindao") of the Shanghai Municipal Science and Technology Commission (grant no.18401902200 to Ling Wang), the Shanghai Municipal Commission of Health and Family Planning Science Foundation for Young Scientists No. 20174Y0224 (to Na Zhang), the Shanghai Committee of The China Democratic League (grant no. 02054 to Ling Wang), the National Natural Science Foundation of China (grant nos. 30801502 and 31571196 to Ling Wang), the 2015 Program to Guide Medicine ("Yixueyindao") of the Shanghai Municipal Science and Technology Commission (grant no. 15401932200 to Ling Wang), the 2008 JSPS Postdoctoral Fellowship for Foreign Researchers (grant no. P08471 to Ling Wang), the Shanghai Pujiang Program (grant no. 11PJ1401900 to Ling Wang), the Shanghai Program for Support of Leading Disciplines-Integrative Medicine (grant no. 20150407), and the Development Project of Shanghai Peak Disciplines-Integrative Medicine no. 20150407.

References

[1] G. Bozdag, S. Mumusoglu, D. Zengin, et al., The prevalence and phenotypic features of polycystic ovary syndrome: a systematic review and meta-analysis, Hum. Reprod. 31 (12) (2016) 2841–2855.
[2] V.G. Thackray, Sex, microbes, and polycystic ovary syndrome, Trends Endocrinol. Metab. 30 (1) (2019) 54–65.
[3] S.B. Sherman, N. Sarsour, M. Salehi, et al., Prenatal androgen exposure causes hypertension and gut microbiota dysbiosis, Gut Microbes 9 (5) (2018) 400–421.
[4] P.J. Torres, D.V. Skarra, B.S. Ho, et al., Letrozole treatment of adult female mice results in a similar reproductive phenotype but distinct changes in metabolism and the gut microbiome compared to pubertal mice, BMC Microbiol. 19 (1) (2019) 57.
[5] K. Tremellen, K. Pearce, Dysbiosis of gut microbiota (DOGMA)–a novel theory for the development of polycystic ovarian syndrome, Med. Hypotheses 79 (1) (2012) 104–112.
[6] X. Qi, C. Yun, L. Sun, et al., Gut microbiota-bile acid-interleukin-22 axis orchestrates polycystic ovary syndrome, Nat. Med. 25 (8) (2019) 1225–1233.
[7] D.A. Dumesic, S.E. Oberfield, E. Stener-Victorin, et al., Scientific statement on the diagnostic criteria, epidemiology, pathophysiology, and molecular genetics of polycystic ovary syndrome, Endocr. Rev. 36 (5) (2015) 487–525.
[8] P.J. Torres, M. Siakowska, B. Banaszewska, et al., Gut microbial diversity in women with polycystic ovary syndrome correlates with hyperandrogenism, J. Clin. Endocrinol. Metab. 103 (4) (2018) 1502–1511.
[9] R. Azziz, E. Carmina, D. Dewailly, et al., Positions statement: criteria for defining polycystic ovary syndrome as a predominantly hyperandrogenic syndrome: an androgen excess society guideline, J. Clin. Endocrinol. Metab. 91 (11) (2006) 4237–4245.
[10] R.L. Rosenfield, The diagnosis of polycystic ovary syndrome in adolescents, Pediatrics 136 (6) (2015) 1154–1165.
[11] C.R. Mccartney, J.C. Marshall, Clinical practice. polycystic ovary syndrome, N. Engl. J. Med. 375 (1) (2016) 54–64.
[12] E.K. Barthelmess, R.K. Naz, Polycystic ovary syndrome: current status and future perspective, Front. Biosci. Elite Ed. (Elite Ed) 6 (2014) 104–119.
[13] V.H. Andrade, A.M. Mata, R.S. Borges, et al., Current aspects of polycystic ovary syndrome: A literature review, Rev. Assoc. Med. Bras. (1992) 62 (9) (2016) 867–871.
[14] R. Dumitrescu, C. Mehedintu, I. Briceag, et al., The polycystic ovary syndrome: an update on metabolic and hormonal mechanisms, J. Med. Life 8 (2) (2015) 142–145.
[15] W. Ratajczak, A. Ryl, A. Mizerski, et al., Immunomodulatory potential of gut microbiome-derived short-chain fatty acids (SCFAs), Acta Biochim. Pol. 66 (1) (2019) 1–12.
[16] L. Lin, J. Zhang, Role of intestinal microbiota and metabolites on gut homeostasis and human diseases, BMC Immunol. 18 (1) (2017) 2.
[17] J.P. Mcaleer, J.K. Kolls, Contributions of the intestinal microbiome in lung immunity, Eur. J. Immunol. 48 (1) (2018) 39–49.
[18] C.A. Woodhouse, V.C. Patel, A. Singanayagam, et al., Review article: the gut microbiome as a therapeutic target in the pathogenesis and treatment of chronic liver disease, Aliment. Pharmacol. Ther. 47 (2) (2018) 192–202.
[19] G.J. Grosicki, R.A. Fielding, M.S. Lustgarten, Gut microbiota contribute to age- related changes in skeletal muscle size, composition, and function: biological basis for a gut-muscle axis, Calcif. Tissue Int. 102 (4) (2018) 433–442.
[20] W. Aw, S. Fukuda, Understanding the role of the gut ecosystem in diabetes mellitus, J. Diabetes Investig. 9 (1) (2018) 5–12.
[21] H. Liu, C. Hu, X. Zhang, et al., Role of gut microbiota, bile acids and their cross-talk in the effects of bariatric surgery on obesity and type 2 diabetes, J. Diabetes Investig. 9 (1) (2018) 13–20.
[22] I. Rowland, G. Gibson, A. Heinken, et al., Gut microbiota functions: metabolism of nutrients and other food components, Eur. J. Nutr. 57 (1) (2018) 1–24.
[23] J.M. Baker, L. Al-Nakkash, M.M. Herbst-Kralovetz, Estrogen-gut microbiome axis: physiological and clinical implications, Maturitas 103 (2017) 45–53.
[24] S.T. Kelley, D.V. Skarra, A.J. Rivera, et al., The gut microbiome is altered in a letrozole-induced mouse model of polycystic ovary syndrome, PLoS One 11 (1) (2016), e0146509.
[25] P.J. Torres, B.S. Ho, P. Arroyo, et al., Exposure to a healthy gut microbiome protects against reproductive and metabolic dysregulation in a pcos mouse model, Endocrinology 160 (5) (2019) 1193–1204.
[26] Y. Guo, Y. Qi, X. Yang, et al., Association between polycystic ovary syndrome and gut microbiota, PLoS One 11 (4) (2016), e0153196.
[27] X.Z. Wan, C. Ai, Y.H. Chen, et al., Physicochemical characterization of a polysaccharide from green microalga chlorella pyrenoidosa and its hypolipidemic activity via gut microbiota regulation in rats, J. Agric. Food Chem. 68 (5) (2020) 1186–1197.
[28] H. Du, A. Zhao, Q. Wang, et al., Supplementation of inulin with various degree of polymerization ameliorates liver injury and gut microbiota dysbiosis in high fat-fed obese mice, J. Agric. Food Chem. 68 (3) (2020) 779–787.
[29] F. Zhang, T. Ma, P. Cui, et al., Diversity of the gut microbiota in dihydrotestosterone-induced pcos rats and the

pharmacologic effects of diane-35, probiotics, and berberine, Front. Microbiol. 10 (2019) 175.
[30] J. Xue, X. Li, P. Liu, et al., Inulin and metformin ameliorate polycystic ovary syndrome via anti-inflammation and modulating gut microbiota in mice, Endocr. J. (2019).
[31] L. Lindheim, M. Bashir, J. Munzker, et al., Alterations in gut microbiome composition and barrier function are associated with reproductive and metabolic defects in women with polycystic ovary syndrome (PCOS): a pilot study, PLoS One 12 (1) (2017), e0168390.
[32] M. Insenser, M. Murri, R. Del Campo, et al., Gut microbiota and the polycystic ovary syndrome: influence of sex, sex hormones, and obesity, J. Clin. Endocrinol. Metab. 103 (7) (2018) 2552–2562.
[33] B. Zeng, Z. Lai, L. Sun, et al., Structural and functional profiles of the gut microbial community in polycystic ovary syndrome with insulin resistance (IR-PCOS): a pilot study, Res. Microbiol. 170 (1) (2019) 43–52.
[34] R. Liu, C. Zhang, Y. Shi, et al., Dysbiosis of gut microbiota associated with clinical parameters in polycystic ovary syndrome, Front. Microbiol. 8 (2017) 324.
[35] J. Zhang, Z. Sun, S. Jiang, et al., Probiotic bifidobacterium lactis v9 regulates the secretion of sex hormones in polycystic ovary syndrome patients through the gut- brain axis, mSystems 4 (2) (2019).
[36] M.X. Maldonado-Gomez, I. Martinez, F. Bottacini, et al., Stable engraftment of bifidobacterium longum ah1206 in the human gut depends on individualized features of the resident microbiome, Cell Host Microbe 20 (4) (2016) 515–526.
[37] P.S. Kumar, Sex and the subgingival microbiome: do female sex steroids affect periodontal bacteria? Periodontology 61 (1) (2000) 103–124, 2013.
[38] M. Soory, Bacterial steroidogenesis by periodontal pathogens and the effect of bacterial enzymes on steroid conversions by human gingival fibroblasts in culture, J. Periodont. Res. 30 (2) (1995) 124–131.
[39] K.B. Singh, D.K. Mahajan, R.P. Tewari, Hormonal modulation of the vaginal bacterial flora in experimental polycystic ovarian disease, J. Clin. Lab. Anal. 10 (5) (1996) 233–238.
[40] A. Chavira, P. Belda-Ferre, T. Kosciolek, et al., The microbiome and its potential for pharmacology, Handb. Exp. Pharmacol. 260 (2019) 301–326.
[41] V. Gopalakrishnan, C.N. Spencer, L. Nezi, et al., Gut microbiome modulates response to anti-PD-1 immunotherapy in melanoma patients, Science 359 (6371) (2018) 97–103.
[42] V. Matson, J. Fessler, R. Bao, et al., The commensal microbiome is associated with anti-PD-1 efficacy in metastatic melanoma patients, Science 359 (6371) (2018) 104–108.
[43] B. Routy, E. Le Chatelier, L. Derosa, et al., Gut microbiome influences efficacy of PD-1-based immunotherapy against epithelial tumors, Science 359 (6371) (2018) 91–97.
[44] N. Iida, A. Dzutsev, C.A. Stewart, et al., Commensal bacteria control cancer response to therapy by modulating the tumor microenvironment, Science 342 (6161) (2013) 967–970.
[45] H.J. Haiser, D.B. Gootenberg, K. Chatman, et al., predicting and manipulating cardiac drug inactivation by the human gut bacterium eggerthella lenta, Science 341 (6143) (2013) 295–298.
[46] A. Nowak, M. Hedenstierna, J. Ursing, et al., Efficacy of routine fecal microbiota transplantation for treatment of recurrent clostridium difficile infection: a retrospective cohort study, Int. J. Microbiol. (2019) (2019), 7395127.
[47] M. Tvede, M. Tinggaard, M. Helms, Rectal bacteriotherapy for recurrent clostridium difficile-associated diarrhoea: results from a case series of 55 patients in denmark 2000-2012, Clin. Microbiol. Infect. 21 (1) (2015) 48–53.
[48] C. Blanchaert, B. Strubbe, H. Peeters, Fecal microbiota transplantation in ulcerative colitis, Acta Gastroenterol. Belg. 82 (4) (2019) 519–528.
[49] Z.K. Wang, Y.S. Yang, Y. Chen, et al., Intestinal microbiota pathogenesis and fecal microbiota transplantation for inflammatory bowel disease, World J. Gastroenterol. 20 (40) (2014) 14805–14820.
[50] W. Zhu, M.G. Winter, M.X. Byndloss, et al., Precision editing of the gut microbiota ameliorates colitis, Nature 553 (7687) (2018) 208–211.
[51] J. Suez, N. Zmora, G. Zilberman-Schapira, et al., Post-antibiotic gut mucosal microbiome reconstitution is impaired by probiotics and improved by autologous fmt, Cell 174 (6) (2018), 1406-23.e16.
[52] W. Song, A.C. Anselmo, L. Huang, Nanotechnology intervention of the microbiome for cancer therapy, Nat. Nanotechnol. 14 (12) (2019) 1093–1103.
[53] V.M. Isabella, B.N. Ha, M.J. Castillo, et al., Development of a synthetic live bacterial therapeutic for the human metabolic disease phenylketonuria, Nat. Biotechnol. 36 (9) (2018) 857–864.
[54] I.Y. Hwang, E. Koh, A. Wong, et al., Engineered probiotic Escherichia coli can eliminate and prevent Pseudomonas aeruginosa gut infection in animal models, Nat. Commun. 8 (2017) 15028.
[55] A. Vrieze, V.E. Nood, F. Holleman, et al., Transfer of intestinal microbiota from 69–75, https://doi.org/10.1016/j.ejogrb.2016.01.040. Epub 2016 Feb 6. PMID: lean donors increases insulin sensitivity in individuals with metabolic syndrome 26901400.
(online first), Gastroenterology 143 (4) (2012).
[56] K.N. Khan, A. Fujishita, H. Masumoto, H. Muto, M. Kitajima, H. Masuzaki, Improved glucose metabolism following bariatric surgery is associated with J. Kitawaki, Molecular detection of intrauterine microbial colonization in women increased circulating bile acid concentrations and remodeling of the gut with endometriosis, Eur. J. Obstet. Gynecol. Reprod. Biol. 199 (April) (2016) microbiome, World J. Gastroenterol. 22 (Oct. (39)) (2016) 8698–8719, https://doi. org/10.3748/wjg.v22.i39.8698. PMID: 27818587; PMCID: PMC5075546.
[57] L. Kaska, T. Sledzinski, A. Chomiczewska, A. Dettlaff-Pokora, J. Swierczynski,